Remarks

Claims 20-24, 32 and 43-57 are pending in the application and stand rejected. The prior rejections of claims 43-57 under 35 U.S.C. § 112, first paragraph and claims 20-24 and 32 under 35 U.S.C.§112, second paragraph have been withdrawn. Applicants acknowledge the Office's recognition that these claims meet the standards required under section 112. The rejection of claims as anticipated by Russ et al. also is withdrawn.

Claims 20-24, 32, 43-45 and 49-56 are rejected under 35 U.S.C. § 103(a) as obvious over Dymecki in view of Von Melchner et al. and Abuin et al. The Office Action describes Dymecki as teaching "methods of site-specific recombination of DNA into the genome of a mammal," using Flp or Cre recombinases, including a two-recombinase system and methods which can inactivate a gene at a specific time or tissue. Dymecki also is cited for teaching that the genetic material can be stably or excisably integrated into its genome and can be transmitted through the germ line to succeeding generations.

With respect to methods for achieving this gene inactivation, Dymecki is cited as teaching that a transgene can have a recombinase operably linked to a regulatory region such as a promoter that is regulated by developmental stage or other factors and that Cre/LoxP can be used to control a series of recombination events. The Office acknowledges that Dymecki does not teach a single transgene flanked by recombinase sites. The claims of this application recite a DNA molecule wherein the transgene is flanked by recombinase sites. Therefore, since Dymecki does not teach the claimed DNA molecule, the Office is relying on Von Melchner et al. for teaching the invention here claimed.

The Office interprets Von Melchner as teaching self-deleting vectors for gene therapy using retroviruses to introduce genes into the mammalian genome. In particular, the Office Action focuses on teachings in Von Melchner et al. that retroviral vectors are the most efficient way to transduce foreign genes into mammalian cells, and that the vector would contain site-specific recombinases, in the same vector or encoded in a separate vector for deletion. The Office Action particularly points to Figure 9A, which assertedly teaches a vector flanked by LoxP sites which has a promoter operably linked to a recombinase gene and a nucleic acid to be deleted.

The Office concludes from this that it would have been obvious for one of skill to use the Von Melchner et al. vector with the techniques of Dymecki with respect to producing transgenic animals in order to excise a gene. The motivation listed in the Office Action is Dymecki's asserted contemplation of removing potentially interfering selectable markers and Von Melchner et al.'s asserted teaching that their vectors can be used to introduce site-specific mutations into the mammalian genome. Additional motivation is asserted to be contained in Abuin et al. with respect to the goal to excise selection markers when producing transgenic mice, including with a Cre/LoxP system.

In order to make out a prima facie case of obviousness, the Office is required to meet all three of the following criteria: (1) the cited art must disclose or suggest each and every element of the rejected claims; (2) there must be motivation in the art to combine and/or modify the fair suggestions of the cited art to achieve the claimed invention; and (3) there must be a reasonable expectation of success that the invention would be achieved by the combination or modification of what is fairly taught or suggested in the cited art. See M.P.E.P. § 2143.

The Office cites Dymecki as the primary reference, however concedes that this references does not teach or suggest a DNA molecule comprising a transgene flanked by recombinase sites, but only teaches various methods for site-specific recombination, use of a recombinase site to engineer a mutation, and use of a recombinase that is operably linked to a regulatory region. In essence, Dymecki is only cited for teaching a method of using a site-specific recombinase to alter genomic DNA, i.e. to produce a transgenic animal.

The Office relies on Von Melchner et al. for teaching the claimed DNA molecule, methods which use the molecule and a transgenic mouse containing it. Applicants submit that the Office's interpretation of the teachings and fair suggestions of Von Melchner et al. is not accurate. Von Melchner et al. do not teach or suggest a DNA molecule wherein a promoter operatively linked to a recombinase gene and the nucleic acid to be removed, flanked by two recombinase sites, in a single nucleotide chain. Applicants have amended independent claims 20, 43 and 49 to further clarify that "the DNA molecule" is a single nucleotide chain.

The Office cites page 2, second full paragraph of Von Melchner, which discloses adaptation of retroviruses for mammalian gene transduction by transfecting vector DNA into cells that contain complete retroviral genomes or helper viruses. The reference stresses the use of retroviral vectors is preferred, but recites a litany of dangers associated with their use in the passage cited by the Office (page 2, line 25-page 3, line 28). The retroviral vector may contain a recombinase. Page 4 of the Von Melchner et al. disclosure specifies that the location for the recombinase-encoding sequence and "the target sequence within the vector" may be chosen by the skilled artisan, and may be in

the same or a different vector from "the target sequence." The skilled artisan would have noted that in each case, "the target sequence" is nowhere taught or suggested to be present in a single vector as two flanking sequences in a single nucleotide chain. See page 4, lines 12, 18-19 and 22.

Von Melchner et al. refer to a "system" in which target-sequence-flanked DNA is excised, for example at page 6, second full paragraph. This "system" involves insertion of two separate vectors into the cell, each of which contains one recombinase site. The vectors subsequently recombine in vivo to duplicate the recombinase site, thereby generating a self-excision construct that contains the two flanking recombinase target sites. Note that on page 6, final paragraph, the specification teaches that the retroviruses of the invention duplicate the target sequences, which enables the recombinase to delete the desired sequence. See page 6, lines 26-30. Thus, the flanking target sequences are only created in vivo and do not exist in the vectors used by Von Melchner. The claimed DNA molecule, on the other hand, is a single piece of DNA that contains the recited components flanked by two recombinase sites, avoiding having to use a two-component system. Figure 5 and the text discussing it on page 11 of Von Melchner show the preferred embodiments, which do not teach the present invention.

Applicants also would like to call attention to Figure 9A, which was cited by the examiner. Figure 8 shows the retrovirus vectors that were actually transfected into the cells (see page 8, lines 26-27 and page 17, lines 1-3) to obtain infectious virus. These vectors do not contain the recited elements of claim 1. The viruses were then used to infect N1H3T3 cells. The predicted structure of the proviruses in vivo is shown in Figure 9A (see page 9, lines 2-3), and then the recombined product,

i.e. the second diagram of Figure 9A in which the sequence already is deleted, is detected. Thus it is clear that Von Melchner never had possession of the DNA which is claimed here, nowhere suggests that such a single DNA molecule would be useful and did not teach how to obtain it. Von Melchner teach a two vector system and not the single DNA molecule claimed here.

Applicants therefore respectfully submit that Von Melchner does not teach or suggest all elements of the claims of this invention, either alone or in combination with Dymecki, which the Office concedes does not teach or suggest this construct. Because the cited art (Abuin is cited for motivation only and also does not disclose or suggest the claimed DNA molecule) does not teach or suggest each and every claim element of the rejected claims, the Office cannot meet the first criterion for making out a prima facie case of obviousness against the pending claims and the rejection should be withdrawn.

The cited references also do not provide any motivation to modify their teachings regarding a two component vector system to achieve what is claimed here. There is no indication that the same results would occur if the vector were produced to comprise a DNA molecule that contains all components of the excision system that are recited in claim 1 here. Although Von Melchner sometimes refer to "at least one" target sequence in their vector system or in cells, the sequence to be deleted and the recombinase are not flanked by two recombinase sites in a single DNA vector because if this were done using the methods of Von Melchner, the nucleic acid between the sites would be excised during vector production, removing the recombinase, whereas the goal in Von Melchner was to insert the material into the mammalian genome and <u>then</u> to excise the desired nucleic acid. The inventive method uses a DNA construct that can be propagated

in bacteria without excision of the cassette, which allows one to synthesize large quantities of pure vector with no premature excision. Von Melchner et al. were forced to use a two-vector reagent to overcome this problem.

It is for this reason that Von Melchner et al. do not provide any suggestion of using a single DNA with two flanking recombinase sites and why there would have been no motivation to change the Von Melchner et al., Dymecki or Abuin et al. teachings to create the invention claimed here and no reasonable expectation of success had it been attempted. The inventive DNA and methods allow one to assemble a single DNA, and make it possible to use naked DNA for gene introduction, for example, rather than a retrovirus.

In summary, Applicants submit that Dymecki and Abuin et al., which only disclose the function of site-specific recombinases and their potential uses in technologies such as gene therapy, e.g. to excise genetic material, combined with the two-vector, duplicating system of Von Melchner et al., do not add up to the single DNA composition and methods which are claimed here. Further, there is nothing to motivate the skilled artisan to modify Von Melchner to create a single DNA method and no reasonable expectation that this approach would be successful. Applicants therefore submit that the Office cannot meet any of the three required criteria for a prima facie case of obviousness and request the rejection be withdrawn.

Claims 46-57 are rejected as obvious over Dymecki in view of Von Melchner et al. and Abuin et al. and further in view of Vidal et al. The additional reference, Vidal et al., is cited for teaching a gamete-specific promoter in production of a transgenic mouse. The other references are discussed above. Vidal et al., like the other cited references, do not teach or suggest the

inventive DNA molecule, containing the recited elements of the claims here presented. Vidal et al. add the element of a gamete-specific promoter, but do not make up for the deficiencies of the references discussed above. Even the combination of all four of the cited references does not teach or suggest all elements of the claims since there is nothing in any or all of the cited art to suggest the recited DNA molecule components, flanked by two recombinase sites, in one DNA molecule. Therefore, the Office cannot meet the first required criterion for making out a prima facie case of obviousness against the claims here.

In summary, the Dymecki, Abuin et al. and Vidal et al. references only discuss methods for using a recombinase such as the Cre-loxP system to create transgenic animals, including methods for spatially restricted promoters and gamete-specific promoters. Von Melchner et al. discuss a self-excision method, but do not teach or suggest a (one) DNA molecule containing a recombinase and a nucleic acid to be deleted, flanked by two recombinase sites. The Office Action refers to "the vector" taught by Dymecki, Von Melchner et al. and Abruin et al., however Applicants submit that the combined references do not teach any self-excision method other than the Von Melchner two-vector system. The remaining references do not even relate to a self-excision vector—they only discuss uses of recombinase to create a mutation in DNA. Nowhere in any of the cited art is the claimed invention taught or even suggested.

It would not be obvious to take the two-vector system of Von Melchner and modify it to a single vector such as Dymecki, Abruin et al. and Vidal et al. used (without a flanked recombinase), because premature excision of the gene would have occurred. This problem does not occur with the inventive method, which employs an intron in the recombinase sequence. Nothing in the art

suggests this method for solving the problems that would occur if the Von Melchner et al. method were modified to a single DNA sequence. Therefore, there is no real motivation in the art to achieve the claims and no reasonable expectation of success absent the hindsight provided by the present inventors' success. Applicants therefore submit that claims 20-24, 32 and 43-57, as well as the new claims added with this amendment are nonobvious over the cited art.

Support for the amendments made to claims 20, 43 and 49 is provided in the specification as a whole, which repeatedly refers to "a DNA molecule" or "the DNA molecule" as containing the elements discussed. Applicants specifically refer to Example 1, which explains construction of "the" cassette, and to Figure 2A and the text relating to this Figure on page 3, lines 7-25. Example 2 teaches use of the vector pRVa3^{ACN}, which is diagramed in Figure 2A as a single nucleotide chain, showing the two recombinase sites (P).

Support for matter need not be verbatim. By disclosing an invention that has a particular function or property, the application necessarily discloses that function or property. Therefore, the application may be amended to recite this property without introducing new matter. See M.P.E.P. §2163.07(a). Applicants submit that the skilled person reading the present patent specification or claims prior to amendment would immediately recognize that the claimed DNA molecule is a single nucleotide chain.

Applicants therefore submit that the amendments do not introduce new matter and do not alter claim scope. The amendments are intended only to clarify that "the DNA molecule comprising" the recited elements is one DNA molecule and not two or more molecules which together contain the recited elements.

Applicants have added new claims 58-63, which are directed to embodiments in which the recombinase gene contains an intron. These claims are supported by the original claims and by the specification as a whole, for example at page 3, lines 10-11; page 6, lines 5-8 and page 9, line 6. The purpose of the intron sequences in the DNA molecule is to prevent in-frame translation and subsequent excision during production in bacteria, allowing one DNA to contain the two flanking recombinase sites and a recombinase.

Applicants submit that the Office cannot make out a prima facie case of obviousness against the present invention and therefore request withdrawal of the rejections based on an assertion of obviousness.

Applicants request reconsideration of the application at this time.

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